Studies on the antimicrobial activity of $\text{Co}_4(\mu_3\cdot\text{O})_4(\mu\cdot\text{O}_2\text{C}.\text{C}_6\text{H}_4\cdot\text{4-Cl})_4(4\cdot\text{Me-Py})_4$ : tetrakis ($\mu_3\cdot\text{oxo}$) tetrakis ($\mu\cdot4\cdot\text{chboro}

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Abstract

Olive-green oxo-bridged Co (III) complex tetrakis($\mu_3\cdot\text{oxo}$) ($\mu\cdot4\cdot\text{chboro}$-benzoato-$\text{O}$) tetrakis (4-methylpyridine) cobalt (III) having the formula $\text{Co}_4(\mu_3\cdot\text{O})_4(\mu\cdot\text{O}_2\text{C}.\text{C}_6\text{H}_4\cdot\text{4-Cl})_4(4\cdot\text{Me-Py})_4$ is synthesized by modification of a reported synthetic method. The synthetic method involves hydrogen peroxide oxidation of a mixture of Co (II), 4-chlorobenzoic acid and 4-methylpyridine in a 1:2:1 molar ratio in methanol. Spectral studies suggest the presence of a distorted cubane-like core of $[\text{Co}_3(\mu_3\cdot\text{O})_4]^{3+}$ in the complex. The complex is found to exhibit inhibitory effect on $\text{Escherichia coli}$ in a dose dependent manner.

Keyword : Cubane cluster, antibacterial, $E.\text{Coli}$.

1. Introduction

Oxo-bridged carboxylate clusters with ancillary N- and O-donor ligands are of continued interest because of their interesting physical properties and varied structural motifs they display (Winpenny et al., 2004, Winpenny et al., 2001, Beattie et al., 1998). Oxo-bridged cluster complexes of cobalt containing a cubane-like core have recently been the subject of extensive studies because of their relevance in various fields viz. materials, catalysis as well as bioinorganic chemistry (Chakrabarty et al., 2007). Cobalt (III) cubane-like clusters of the type $\text{Co}_2\text{O}_4(\text{O}_2\text{CR})_4\text{L}_4$, where R is a methyl or an aryl group and L is either a pyridine or a substituted pyridine ligand, play active role in the catalyzed oxidation of organic substrates such as alkylaromatics, alcohols, and $\infty$-pinene, with air, oxygen, or TBHP (tetrabutyl hydroperoxide) as the oxidants (Chakrabarty et al., 2007, Chakrabarty et al., 2007, Sarmah et al., 2007, Chakrabarty et al., 2004, Chakrabarty, 2009, Chakrabarty et al., 2009). The $[\text{Co}_3\text{II}\text{CoIV}(\mu_3\cdot\text{O})_4]^{5+}$ core present in these complexes undergo a reversible one-electron oxidation to the $[\text{Co}_3\text{III}\text{CoIV}(\mu_3\cdot\text{O})_4]^{5+}$ core (Chakrabarty et al., 2007, Chakrabarty et al., 2009, Chakrabarty et al., 2009, Chakrabarty et al., 2007)

During the past decades, much attention has been given to the synthesis of new metal complexes and the evaluation of these agents for antibacterial activity (Aliyu et al., 2012, Chang et al., 2010, Saha et al., 2009). This revival interest was generated by the discovery of the antibacterial, antifungal and anticancer activity of several metal complexes. In the last few years so many studies has been done on the structure and chemical behaviour of several metal complexes to find out an alternative against the drugs. The antimicrobial activity of Cobalt complex against the multi drug resistant organism is very scantly; the present study is an attempt to understand antimicrobial activity of Cobalt complex (Aliyu et al., 2012). More recently, cobalt (III) based ligand complexes have been found to possess both

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antiviral and antibacterial activities (Aliyu et al., 2012, Chang et al., 2010, Saha et al., 2009). Because of their suitability as redox catalysts for the oxidation of various organic substrates these complexes are also expected to show some antimicrobial property (Beattie et al., 1998). The present work stems from our interest to study the antimicrobial activity of redox active cobalt (III) complex. The interesting finding of the study is the significant antimicrobial activity displayed by the complex.

2. Experimental Section

2.1 Materials

All chemicals used were obtained from commercial sources and without any further purification. Co(NO₃)₂.6H₂O, hydrogen peroxide were procured from E. Merck (India), 4-methylpyrididine E. Merck (Germany). The solvent methanol used for synthesis was of reagent grade. Dichloromethane, acetonitrile, DMSO and methanol used for spectral electrochemical measurements and antimicrobial study were of HPLC grade. One gram negative microbial strain viz: Escherichia coli (gram negative) were obtained from the Institutional Biotech. Hub, Karimganj College and grown on nutrient broth (Hi-media) at 37°C in an incubator for 18-24 hrs and maintained on respective agar slant at 4°C. The standard disc-diffusion method was used to evaluate the antimicrobial activity.

2.2 Measurements

Infrared spectrum of the complex was recorded in KBr pellets using a Perkin-Elmer RX1 FT-IR spectrophotometer in the mid-IR region (4000-450 cm⁻¹). Perkin-Elmer Lambda 40 spectrophotometer is used to record the electronic spectra of the complex. Cobalt analysis was performed gravimetrically by precipitating out cobalt as Hg[Co(NCS)₄] following standard procedure. (Chakrabarty et al., 2007) Cyclic and differential pulse voltammetric measurements were performed using an EG&G PAR model 253 VersaStat potentiostat/galvanostat having a three-electrode setup consisting of a glassy-carbon working, platinum-wire auxiliary, and a saturated calomel reference electrode (SCE). (Chakrabarty et al., 2009, Chakrabarty et al., 2007)

2.3 Preparation

The complex was synthesized by following a reported procedure in a modified form (Chakrabarty et al., 2009). A mixture of Co(NO₃)₂.6H₂O (2.91 g, 10 mM) and sodium-4-chlorobenzoate (3.573 g, 20 mM) in methanol (20 mL) was stirred. 4-methylpyridine (0.98 mL, 10 mM) was added to the reaction mixture and refluxed. 30% (v/v) hydrogen peroxide solution (5 mL, ~50 mM) was then added slowly. The reaction mixture was stirred under refluxing condition for 4 h. Olive green compound (~0.47 g) precipitated out during the reaction. The cooled reaction mixture was concentrated in a rotary-evaporator and the aqueous layer was separated out by adding dichloromethane to it. An olive green compound precipitated out on addition of petroleum ether (40-60 fractions) to the dichloromethane solution. (Chakrabarty et al., 2009). The solid was dried in a vacuum desiccator over fused CaCl₂. Yield : 1.97 g (66.7% based on cobalt).

$$\text{Co(NO}_3\text{)}_2\cdot 6\text{H}_2\text{O} + 4\text{-Cl-}C_6\text{H}_4\text{-COONa} + 4\text{-CH}_3\text{-C}_6\text{H}_4\text{N} \xrightarrow{\text{CH}_3\text{OH}} \text{Pink Solution}$$

$$\text{H}_2\text{O}_2(30\% \text{ V/V}), \text{Reflux} \xrightarrow{4\text{hr}} \text{Co}_4(\mu_3\text{-O})_4(\mu_2\text{-O}_2\text{C}C_6\text{H}_4\text{-4-Cl})_4(4\text{-Me-Py})_4 \xrightarrow{\text{Pink Solution}} \text{Olive Green ppt.}$$
2.4 Characterisation

2.4.1 Infrared Spectra

The complex has been characterized by infrared spectroscopy in KBr phase in the mid-IR region (4000 – 450 cm$^{-1}$). In the IR spectra, it is possible to identify bands due to bridging carboxylate anions as well as for the N-donor pyridine ligand. The IR-stretching frequency of the complex in cm$^{-1}$ is shown below.

<table>
<thead>
<tr>
<th>$\nu_{\text{asym}}$ (COO$^-$) cm$^{-1}$</th>
<th>$\nu_{\text{sym}}$ (COO$^-$) cm$^{-1}$</th>
<th>pyridyl ring stretches cm$^{-1}$</th>
<th>$\mu_3$-oxo-bridge cm$^{-1}$</th>
<th>-CH stretch cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1542</td>
<td>1392</td>
<td>1625, 1512, 1494</td>
<td>636</td>
<td>2930, 2826</td>
</tr>
</tbody>
</table>

2.4.2 Electronic Spectra

The UV-visible spectrum for the complexes is recorded in CH$_2$Cl$_2$ at different concentrations. The bands in CH$_2$Cl$_2$ (a) $10^{-3}$ M occur at 633 nm, and (b) $10^{-4}$ M occur at 362 nm and 246 nm. The spectra are slightly concentration-dependent. The lowest energy band corresponds to the d-d transition involving either $1A_1 \rightarrow 1T_1$ or $1A_1 \rightarrow 1T_2$ for the approximately octahedral low-spin $d^6$ Co(III) centers (Chakrabarty et al., 2009, Chakrabarty et al., 2007, Aliyu et al., 2012). The other two bands can be attributed to ligand to metal charge transfer transitions (LMCT). While the first LMCT band at 362 nm is expected to be due to a transition involving the $\mu_3$-O-Co (III) moiety present in the complex (Chakrabarty et al., 2009), later at 246 nm most probably involves a transition between a molecular orbital from the carboxylato ligands and a vacant cobalt (III) $e_g^*$ orbital (Chakrabarty et al., 2007). The uv-visible spectra of the complex in $\lambda_{\text{max}}$ (CH$_2$Cl$_2$), nm is given below:
2.4.3 Cyclic Voltammetry

The complex is found to be electrochemically active. The complex undergoes a one-electron oxidation. The stoichiometry of the electron transfer indicates that the process involves one electron oxidation of the cubane core from [Co₄(µ³-O)₄]⁴⁺ to [Co₃Co⁴(µ³-O)₄]⁵⁺. (Chakrabarty et al., 2009, Chakrabarty et al., 2007, Aliyu et al., 2012). The complex also display well-shaped nearly reversible redox behaviour. The observed \( E_{1/2} \) and \( ΔE_{p} \) values for the complex are found to be 0.83 V and 172 mV respectively while the DPV peak potentials ranges from 0.84 V. Cyclic voltammogram for the complex in CH₂Cl₂-0.1M TBAP vs. SCE. (scan rate of 20 mVs⁻¹) is shown below.

![Cyclic Voltammogram](image)

2.5 Antimicrobial study

The chemicals were dissolved in DMSO in eppendorf tubes at the concentration of 0.01mg/ml, 0.05mg/ml and 0.1mg/ml and sterile paper discs were made from Whatman No.1 filter paper and soaked in the respective solutions for \( \frac{1}{2} \) an hour and then left in laminar airflow hood overnight for drying. Bacteria were cultured over night at 37°C in nutrient agar (NA) media and used as inoculums. Using the standard disc-diffusion method with sterilized glass spreader, the petridishes containing nutrient agar were seeded. Prepared discs of 6 mm in diameter (Whatman No-1 filter paper) containing the compound were placed on seeded nutrient agar plates. Tetracycline discs were used as positive control. The organic solvents were used as negative control. Test plates were incubated at 37°C for 18-24 hours. Antimicrobial activity was evaluated by measuring the zone of inhibition against E. coli mean value. The experiments were conducted in triplicates and the data was tabulated.

![Antimicrobial Zone](image)

Figure-1 : Antimicrobial Zone formed by the compound against E. coli. (1) + control. (2)0.1mg/ml. (3) 0.05mg/ml. (4) 0.01mg/ml. (5) – control.
2.5.1 Result (Antimicrobial Assay)

<table>
<thead>
<tr>
<th>Compounds used</th>
<th>Concentration used</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-complex</td>
<td>0.1mg/ml</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>0.05mg/ml</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>0.01mg/ml</td>
<td>7</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Standard</td>
<td>17</td>
</tr>
<tr>
<td>DMSO</td>
<td>Standard</td>
<td>6</td>
</tr>
</tbody>
</table>

Table-1 : Table showing the concentration of the Co-complexes and the zone of inhibition against *Escherichia coli* (microorganism).

3. Discussion

The IR, electronic spectral study and cyclic voltammetry for the complex indicate that the complex is found to be identical with the reported compound (Chakrabarty, 2009) although the single crystal structure is yet to be obtained. For one Gram negative bacteria viz. *Escherichia coli* it was seen that the compound had significant inhibitory effects on the microbe. The result obtained from the experiment with the Co-complex was analysed and found that the Inhibitory zone of 11.0 mm was exhibited by the compound at a concentration of 0.1mg/ml followed by a zone of 8.0mm at a concentration of 0.05mg/ml. It can be inferred that the compound was not effective at low concentration. Though in comparison to tetracycline, the zone formed was smaller, but it can be assumed that if the concentration of the compound is increased then, the potency of the antimicrobial activity can also be increased. The effect of the complex on gram positive bacteria was found to be insignificant.

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